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AND TRADEMARK OFFICE**

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Applicant : Makoto KOIZUMI

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For : OLIGONUCLEOTIDES HAVING A 2'-O,4'-C-
ETHYLENE NUCLEOTIDE IN THE THIRD POSITION

Art Unit : 1637

Examiner : Mark STAPLES

Docket No. : 06189/HG

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DECLARATION UNDER 37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

MAIL STOP AMENDMENT

SIR :

I, Makoto Koizumi, the inventor of the above-identified application, declare as follows:

A. My Education and Experience

1. I graduated from Hokkaido University, Sapporo, Japan, in the year 1986, and I received a doctorate degree from Hokkaido University on the studies of catalytic ribozymes with sequence-specific RNA cleaving activity in 1991.

2. I have worked for Sankyo Company, Limited, Tokyo, Japan, since 1991. My research activities at Sankyo Company, Limited have included the following: synthesis of nucleoside analogs with antibiotic activity and synthesis of modified oligonucleotides with antivirus, anticancer and anti-diabetes activity. My research activities were not limited to those in the company; I studied as a visiting researcher at Yale University, New Haven, CT, for two years from November 1997. I presently hold the position of the Chief Researcher of Core Technology Research Laboratories of Sankyo Company, Limited. I have held the position of the Senior Researcher of Exploratory Research Laboratories I of Daiichi Sankyo Company, Limited since the year 2007.

3. I am a member of the Pharmaceutical Society of Japan. I am on the committee of Antisense DNA/RNA Society, Japan.

4. I have contributed many scientific papers. For example, I am a co-author of "Biologically active oligodeoxyribo-nucleotides. 5. 5'-End-substituted d(TGGGAG) possesses anti-human immunodeficiency virus type 1 activity by forming a G-quadruplex structure." *J. Med. Chem.* 1998, 41, 3655-3663; "Allosteric selection of ribozymes that respond to the second messengers cGMP and cAMP." *Nat. Struct. Biol.* 1999, 6, 1062-1071. Also, I am a corresponding author of "Synthesis and properties of 2'-O,4'-C-ethylene-bridged nucleic acids (ENA) as effective antisense oligonucleotides." *Bioorg. Med. Chem.* 2003, 11, 2211-2226; "Triplex formation with 2'-O,4'-C-ethylene-bridged nucleic acids (ENA) having C3'-endo conformation at physiological pH." *Nucleic Acids Res.* 2003, 31, 3267-3273; "Direct comparison of in vivo antisense activity of ENA oligonucleotides targeting PTP1B mRNA with that of 2'-O-(2-methoxy)ethyl-modified oligonucleotides." *Oligonucleotides* 2006, 16, 253-262.

5. I am named as an inventor of many patents issued in Japan, the United States and other countries. I am listed as an inventor in the following US patents: "Looped hairpin ribozyme." U.S. Patent 5,631,115 (issued May 20, 1997); "Modified oligodeoxyribonucleotides." U.S. Patent 5,674,856 (issued October 7, 1997);

"Composition and method for the treatment or prophylaxis of viral infections using modified oligodeoxyribonucleotides." U.S. Patent 5,807,837 (issued September 15, 1998).

6. I was the recipient of the following academic and professional awards: "JB award, the Japanese Biochemical Society, 1996"; "Bioorganic Medicinal Chemistry Most Cited Paper 2003-2006 Award, 2006".

7. I am an expert in the way and manner of selecting and synthesizing compounds suitable for development as pharmaceuticals based on the chemistry and biology of nucleosides, nucleotides and oligonucleotides.

B. I am providing my opinion herein concerning the level of skill of a person of ordinary skill in the art of designing and synthesizing pharmaceutical compounds.

1. A person of ordinary skill in the art is a hypothetical person who is presumed to be aware of all the pertinent prior art.

2. A person of ordinary skill in the art is a person to whom an expert in the art could assign a task of moderate difficulty, with reasonable assurance that the task would be accomplished without a great deal of supervision.

3. The following factors should be considered in determining who is a person of ordinary skill in the art: the types of problems encountered in the art; the prior art solutions to those problems; the rapidity with which innovations are made; the sophistication of the technology; and the educational level and experience of active workers in the field.

4. A person with a graduate degree, such as a Master's Degree or a Doctorate Degree, in a field requiring knowledge of organic chemistry and/or biochemistry. The person with a Master's Degree should have at least five years research experience. The

person with a Doctorate Degree should have at least two years of post-graduate academic or two years of post-doctoral research in a company. The research experience of such a person should be in working for a pharmaceutical company in the development of pharmaceuticals to achieve related pharmaceuticals or medical organic compounds having the same or enhanced properties.

C. The following statements of fact and opinion hereinafter, in my opinion, would be the statements of fact and opinion of a person of ordinary skill in the art identified hereinbefore.

1. Latorra et al., Human Mutations, (2003), 22, 79-85 (hereinafter referred to as "Latorra et al.") was cited as the primary reference in three rejections under 35 USC 103 in the August 20, 2008 Office Action in the above-identified application. A secondary reference in all of said three rejections under 35 USC 103 was a publication on which I am a coauthor, namely, Koizumi et al., Nucleic Acids Research, (2003), 13, No. 12, 3267-3273 (hereinafter referred to as the "Koizumi et al. publication").

2. The left and right columns of page 80 of Latorra et al. refer to a locked nucleic acid (LNA) fixed at the 3' terminal position.

3. On page 4 of the aforesaid August 20, 2008 Office Action, reference was made to the legend of Table 1 on page 81 of Latorra et al., wherein the following is stated:

"A total of 16 forward DNA and 3' LNA primers were designed for each of three pUC19 targets, and included match and the three other possible mismatch combinations at the last four positions of each 3' end."

4. It is respectfully submitted that a person of ordinary skill in the art would not consider that the above sentence teaches placing a LNA at the third position from the 3' end for the following reasons:

In the left column, lines 20 to 24 on page 80 of Latorra et al, the following is

stated:

"In this report we investigated the specificity and sensitivity of AS-PCR with primers containing a single locked nucleic acid (LNA) base at the 3' terminal position (referred to as LNA primer)."

In the right column, lines 17 to 20 on page 81 of Latorra et al, the following is stated:

"Matched DNA and LNA primers were designed for three target sequences in pUC19 having A, C, G or T at the position corresponding to the 3' end, along with all possible 3' mismatched primer (see Table 1 legend)."

The above two sentences mean that Latorra et al made primers containing a LNA at the 3' terminal position and with all possible matched and mismatched base pairs at the 3' terminal position.

As discussed above, the Latorra et al. LNA primer design is stated in the legend of Table 1 on page 81 of Latorra et al. as follows:

"A total of 16 forward DNA and 3' LNA primers were designed for each of three pUC19 targets, and included match and the three other possible mismatch combinations at the last four positions of each 3' end."

Furthermore, Latorra et al explained the design of the LNA primers in detail in the next two sentences in the legend of Table 1 on page 81 of Latorra et al., as follows:

"The core sequence shown is the furthest perfect match to the right of the final base sequence. Three other matched forward primers (each ending with a different base) were made by staggering one base to the left from this core sequence and maintaining a constant length (18nt)."

From the above sentences, the following describes concrete sequences for LNA primers that Latorra et al. could have used in their experiments, using Forward primer 1 in Table 1 on page 81 of Latorra et al., as an example. In the case of Forward primer 1, the core structure is GCAGGCCTTCGCTATTACG. The corresponding base to the 3' end of the primer is C in the template of pUC19.

Forward primer 1 GCAGGCCTTCGCTATTACG (primer:template G:C, matched)

Forward primer 1 with a mismatched base at the 3' end can be described as follows:

Forward primer 1A: GCAGGCCTTCGCTATTAC~~A~~ (primer:template A:C, mismatched)

Forward primer 1C: GCAGGCCTTCGCTATTACC (primer:template C:C, mismatched)

Forward primer 1T: GCAGGCCTTCGCTATTACT (primer:template T:C, mismatched)

The other matched primer can be designed by staggering one base to the left from this core sequence (referred to as Forward primer 1-1). G at the 3' end of Forward primer 1 is deleted and a nucleotide is added at the 5' end. However, the additional nucleotide sequence is unknown from the Latorra et al. reference. Here this additional nucleotide is described as N. In this case, the corresponding base to the 3' end of the primer is G in the template of pUC19. The chain length should be maintained as 18nt.

Forward primer 1-1: NGCAGGCCTTCGCTATTAC (primer:template C:G, matched)

Furthermore, Forward primer 1-1 with a mismatched base at the 3' end can be described as follows:

Forward primer 1-1A: NGCGGGCCTTCGCTATTAA (primer:template A:G, mismatched)

Forward primer 1-1G: NGCGGGCCTTCGCTATTAG (primer:template G:G, mismatched)

Forward primer 1-1T: NGCGGGCCTTCGCTATTAT (primer:template T:G, mismatched)

The other matched primer can be designed by staggering one base to the left from Forward primer 1-1 (referred to as Forward primer 1-2). C at the 3' end of Forward primer 1-1 is deleted and a nucleotide is added at the 5' end. However, the additional nucleotide sequence is unknown from the Latorra et al. reference. Here this additional nucleotide is described as N'. In this case, the corresponding base to the 3' end of the primer is T in the template of pUC19. The chain length should be maintained as 18nt.

Forward primer 1-2: N'NGCGGGCCTTCGCTATT (primer:template A:T, matched)

Furthermore, Forward primer 1-2 with a mismatched base at the 3' end can be described as followed.

Forward primer 1-2G: N'NGCGGGCCTTCGCTATTG (primer:template G:T, mismatched)

Forward primer 1-2C: N'NGCGGGCCTTCGCTATTC (primer:template C:T, mismatched)

Forward primer 1-2T: N'NGCGGGCCTTCGCTATTT (primer:template T:T, mismatched)

The other matched primer can be designed by staggering one base to the left from Forward primer 1-2 (referred to as Forward primer 1-3). A at the 3' end of Forward primer

1-2 is deleted and a nucleotide is added at the 5' end. However, the additional nucleotide sequence is unknown from the Latorra et al. reference. Here this additional nucleotide is described as N". In this case, the corresponding base to the 3' end of the primer is A in the template of pUC19. The chain length should be maintained as 18nt.

Forward primer 1-3: N"N'NGCGGGCCTTCGCTATT (primer:template T:A, matched)

Furthermore, Forward primer 1 -3 with a mismatched base at the 3' end can be described as follows:

Forward primer 1-3A: N"N'NGCGGGCCTTCGCTATA... (primer:template A:A, mismatched)

Forward primer 1-3G: N"N'NGCGGGCCTTCGCTATG (primer:template G:A, mismatched)

Forward primer 1-3C: N"N'NGCGGGCCTTCGCTATC (primer:template C:A, mismatched)

I have described above 16 primers with all possible matched and mismatched bases at the 3' end using Forward primer 1 as the core sequence according to the Latorra et al. reference. The same process was performed for Forward primers 2 and 3 to obtain the following two sets of 16 primers with all possible matched and mismatched bases by Latorra et al.

For Forward primers 2:

Forward primer 2 GCGAAAGGGGGATGTGCTGCA (primer:template A:T, matched)

Forward primer 2G GCGAAAGGGGGATGTGCTGCG (primer:template G:T, mismatched)

Forward primer 2C GCGAAAGGGGGATGTGCTGCC (primer:template C:T, mismatched)

Forward primer 2T GCGAAAGGGGGATGTGCTGCT (primer:template T:T, mismatched)

Forward primer 2-1: NGCGAAAGGGGGATGTGCTGC (primer:template C:G, matched)
Forward primer 2-1A: NGCGAAAGGGGGATGTGCTGA (primer:template A:G, mismatched)
Forward primer 2-1G: NGCGAAAGGGGGATGTGCTGG (primer:template G:G, mismatched)
Forward primer 2-1T: NGCGAAAGGGGGATGTGCTGT (primer:template T:G, mismatched)
Forward primer 2-2: N'NGCGAAAGGGGGATGTGCTG (primer:template G:C, matched)
Forward primer 2-2A: N'NGCGAAAGGGGGATGTGCTA (primer:template A:C, mismatched)
Forward primer 2-2C: N'NGCGAAAGGGGGATGTGCTC (primer:template C:C, mismatched)
Forward primer 2-2T: N'NGCGAAAGGGGGATGTGCTT (primer:template T:C, mismatched)
Forward primer 2-3: N"N'NGCGAAAGGGGGATGTGCT (primer:template T:A, matched)
Forward primer 2-3A: N"N'NGCGAAAGGGGGATGTGCA (primer:template A:A, mismatched)
Forward primer 2-3G: N"N'NGCGAAAGGGGGATGTGCG (primer:template G:A, mismatched)
Forward primer 2-3C: N"N'NGCGAAAGGGGGATGTGCC (primer:template C:A, mismatched)

For Forward primers 3:

Forward primer 3 GGGGTGCCTAATGAGTGAGCT (primer:template T:A, matched)
Forward primer 3A GGGGTGCCTAATGAGTGAGCA (primer:template A:A, mismatched)
Forward primer 3G GGGGTGCCTAATGAGTGAGCG (primer:template G:A, mismatched)
Forward primer 3C GGGGTGCCTAATGAGTGAGCC (primer:template C:A,

mismatched)

Forward primer 3-1: NGGGGTGCCTAATGAGTGAGC (primer:template C:G, matched)

Forward primer 3-1A: NGGGGTGCCTAATGAGTGAGA (primer:template A:G, mismatched)

Forward primer 3-1G: NGGGGTGCCTAATGAGTGAGG (primer:template G:G, mismatched)

Forward primer 3-1T: NGGGGTGCCTAATGAGTGAGT (primer:template T:G, mismatched)

Forward primer 3-2: N'NGGGGTGCCTAATGAGTGAG (primer:template G:C, matched)

Forward primer 3-2A: N'NGGGGTGCCTAATGAGTGAA (primer:template A:C, mismatched)

Forward primer 3-2C: N'NGGGGTGCCTAATGAGTGAC (primer:template C:C, mismatched)

Forward primer 3-2T: N'NGGGGTGCCTAATGAGTGAT (primer:template T:C, mismatched)

Forward primer 3-3: N" N'NGGGGTGCCTAATGAGTGTA (primer:template A:T, matched)

Forward primer 3-3G: N" N'NGGGGTGCCTAATGAGTGG (primer:template G:T, mismatched)

Forward primer 3-3C: N" N'NGGGGTGCCTAATGAGTGC (primer:template C:T, mismatched)

Forward primer 3-3T: N" N'NGGGGTGCCTAATGAGTGT (primer:template T:T, mismatched)

As described above, a person of ordinary skill in the art would consider that Latorra et al. used these primers containing a LNA at the 3' terminal position and with all possible matched and mismatched base pairs at the 3' terminal position. Therefore, a person of ordinary skill in the art would not consider to place a LNA at the third position from the 3' end based on the disclosure of Latorra et al.

5. It is respectfully submitted that a person of ordinary skill in the art would not consider to substitute the ENA units of Koizumi et al. for the LNA units of Latorra et al. for the following reasons:

ENA units are considered to be superior to LNA units in triplex formation as described in Koizumi et al. This means that triplex forming oligonucleotides (TFOs) containing ENA units can tightly bind to double-strand DNA to form triplexes compared to TFOs containing LNA units. In Koizumi et al., molecular interaction between double-strand DNA and modified oligonucleotides such as ENA and LNA is discussed.

Molecular interaction between double-strand DNA and TFO is described in Chapter 27, line 1 on page 487 of "Applied Antisense Oligonucleotide Technology," edited by C.A. Stein and Arthur M. Krieg as follows:

"Triple helix formation can be mediated by binding of selected oligonucleotides to homopurine regions of the duplex DNA. These triplex-forming oligonucleotides (TFO) bind specifically in the major groove of the DNA, forming hydrogen bonds with bases in purine-rich strands."

However, in the above-identified patent application, I disclosed how DNA polymerase recognizes double-strand DNA with a template and a primer and elongates a stand. It is a molecular interaction between a protein and a double-strand DNA modified with ENA.

Molecular interaction between protein and double-strand DNA is described in the Summary on page 345, "Nucleic Acids in Chemistry and Biology," edited by G. Michael Blackburn and Michael J. Gait as follows:

"All nucleic acids have repeating polyanionic backbones, and so all proteins that bind to nucleic acids have strategically

placed arginines and lysines that create an electrostatic field to neutralize the negative charge. To interact with B-DNA, the protein either (1) inserts an alpha-helix into the major groove, or (2) inserts a beta-sheet into the minor groove, and form hydrogen-bonds from the side-chains to specific bases."

The disclosure in Koizumi et al. involving a molecular interaction between double-strand DNA and modified oligonucleotides such as ENA and LNA, is completely and qualitatively different from the above-identified application, which concerns a molecular interaction between a protein and a double-strand DNA modified with ENA. Therefore, one of ordinary skill in the art would not consider substituting the ENA units of Koizumi et al. for the LNA units of Latorra et al.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: Oct / 29 / 2008



Makoto KOIZUMI

APPLIED ANTISENSE OLIGONUCLEOTIDE TECHNOLOGY

EDITED BY
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CHAPTER 27

TRIPLEX-FORMING OLIGONUCLEOTIDES FOR GENETIC MANIPULATION: AN ALTERNATIVE VIEW

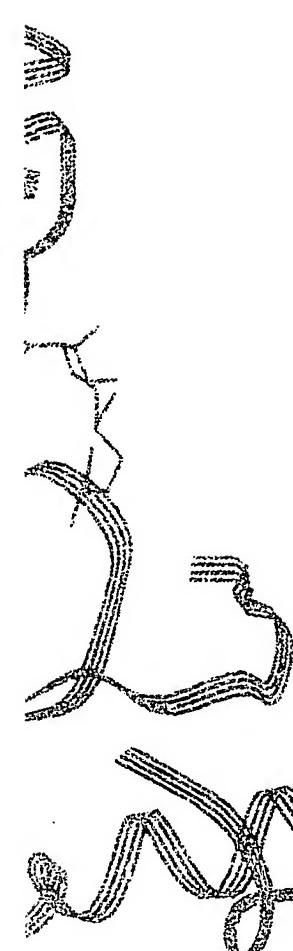
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27.1. INTRODUCTION

Triple helix formation can be mediated by the binding of selected oligonucleotides to homopurine regions of the duplex DNA. These triplex-forming oligonucleotides (TFOs) bind specifically in the major groove of the DNA, forming hydrogen bonds with bases in the purine-rich strand. In the pyrimidine motif, a homopyrimidine oligonucleotide binds in a direction parallel to the purine strand in the duplex through Hoogsteen hydrogen bonds (Moser and Dervan, 1987; Praseuth et al., 1988). In the purine motif, the TFO binds antiparallel to the purine strand in the Watson-Crick duplex via reverse Hoogsteen hydrogen bonds, with A or T binding to A:T and G binding to G:C base pairs (Beal and Dervan, 1991).

The ability of TFOs to bind tightly to DNA in a sequence-specific manner has provided a powerful tool for genetic manipulation. Oligonucleotides designed to bind to sites in gene promoters have been used to block DNA binding proteins and to inhibit transcription both *in vitro* and *in vivo* (Duval-Valentin et al., 1992; Maher et al., 1989). The conjugation of oligonucleotides to crosslinking agents has been utilized to enhance the stability of triplex binding via covalent bond formation in experiments designed to prevent transcription initiation or elongation in plasmid constructs (Praseuth et al., 1988; Sun et al., 1989; Takasugi et al., 1991; Young et al., 1991). Levels of expression of endogenous genes in cells in culture have also been manipulated using TFOs (Blume et al., 1992; Orson et al., 1991; Postel et al., 1991). In addition, TFOs, coupled to DNA cleaving agents, have been used to mediate site-specific cleavage of DNA *in vitro* (Francois et al., 1989a,b; Moser and Dervan, 1987; Perrouault et al., 1990; Strobel et al., 1991). This technique could have potential applications in chromosome mapping.



Nucleic Acids in Chemistry and Biology

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9.1.5 The kinetics of forming protein–nucleic acid complexes

Two factors affect the rate of formation of protein–nucleic acid complexes that are not sequence-specific: random thermal diffusion and long-range, directional electrostatic attraction. They also affect the rate of formation of sequence-specific protein–nucleic acid complexes, but are not enough to account for the observed rates of formation. These are far faster than those predicted from the simplest model of random association of the protein on the nucleic acid, followed by dissociation and reassociation elsewhere if the sequence is not correct. This model is a three-dimensional random walk through the contents of the entire cell, and is clearly too slow.

An alternative model, which can account for the observed rate of formation of sequence-specific protein–DNA complexes, is a one-dimensional random walk. The protein first binds non-specifically to the DNA, and then diffuses or jumps along the DNA until it finds the appropriate sequence. How a protein which normally binds specifically can initially bind non-specifically is not clear. There must be relatively strong interactions which are *not* sequence-specific. The sequence-specific interactions to double-stranded DNA may be hydrogen-bonds between the protein and the DNA. While the protein is bound non-specifically ~~these hydrogen-~~ bonds cannot form, thus decreasing the stability of the non-specific complex. Alternatively, as in *lac* repressor, more ionic interactions may occur in the non-specific complex. Therefore such proteins may well exist in two conformations. The non-specific binding conformation would increase the rate of diffusion along the DNA, and the specific binding conformation, where hydrogen-bonds can be made to the bases, would form only when the correct DNA sequence is reached.

Summary

All nucleic acids have repeating polyanionic backbones, and so all proteins that bind to nucleic acids have strategically placed arginines and lysines that create an electrostatic field to neutralize the negative charge. To interact with B-DNA, the protein either (1) inserts an α -helix into the major groove, or (2) inserts a β -sheet into the minor groove, and forms hydrogen-bonds from the side-chains to specific bases. To interact with single-stranded nucleic acids, aromatic side-chains are used to stack against the nucleic acid bases.

Sequence-specific protein–nucleic acid complexes must be formed by the protein first binding loosely to the incorrect sequence and then diffusing along the DNA in a one-dimensional random walk until it finds the correct sequence. Thus all sequence-specific nucleic acid-binding proteins may exist in two conformations: one that allows tight, sequence-specific binding and one that allows looser, non-sequence-specific binding.